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Cloning of MYB28 Gene from Arabidopsis Thaliana and It's Over Expression in Agrobacterium Tumefaciens Mediated Genetically Transformed Arabidopsis Thaliana Plant from Vindhya Region of Madhya Pradesh

Rajneesh Sharma[1*], Prof. Rashmi Arnold[2]
[1]*Centre for Biotechnology and Microbiology Studies, Awadhesh Pratap Singh University, Rewa, (Madhya Pradesh), 486003*
[2]*Department of Botany, Government Girls Post Graduate College, Rewa (Madhya Pradesh), 486001*

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ABSTRACT
The MYB28 gene, a pivotal and multifaceted regulator of glucosinolates biosynthesis in Arabidopsis thaliana, stands as a beacon illuminating the intricate landscape of plant defense mechanisms. The harnessing of its remarkable potential for diverse research avenues and biotechnological applications frequently necessitates its cloning and expression in an array of host organisms. In this comprehensive article, we embark on an exploration of the remarkably versatile approach of cloning and expressing MYB28 in both the microbial workhorse Escherichia coli (E. coli) and the plant transformation powerhouse, Agrobacterium species. Through this dual strategy, we shed light on the profound significance and myriad applications that emerge at the intersection of these two host organisms and the MYB28 gene.

Keywords: YB28 Gene, Arabidopsis thaliana, E. Coli, Gene Cloning

I. INTRODUCTION
The Myb family of transcription factors includes the MYB28 gene, that is crucial regarding the production of secondary metabolites that include sulfur called glucosinolates that have important defense implications for plants (Hirai, Sugiyama et al. 2007, Yatusevich 2008). Its role as a master regulator of glucosinolate biosynthesis has captured the keen interest of the scientific community. In pursuit of expanding horizons for crop protection and elevating nutritional value, researchers and biotechnologists are fervently exploring ways to unlock the immense potential residing within MYB28. This endeavor often leads to the cloning and expression of MYB28 in a multitude of host organisms, including the versatile E. coli and the transformative Agrobacterium species, opening up a diverse spectrum of pathways for profound investigation and innovative application. Present study will also investigate the expression level of MYB28 gene in transgenic A. thaliana plant and chlorophyll content in transgenic verses wild type A. thaliana plant (Neequaye, Steuernagel et al. 2022).

II. MATERIALS AND METHODS
Cloning and expressing the MYB28 gene from Arabidopsis thaliana in DH5α cell line (E. coli).

The MYB28 gene from Arabidopsis thaliana is cloned in DH5α (E. coli) in a number of steps. The National Center for Biotechnology Information (NCBI) and the Arabidopsis Database (https://www.arabidopsis.org/) provided the genomic DNA sequence. Using the following gene-specific primers, PCR amplification was carried out on A. thaliana cDNA (Garcia-Hernandez, Berardini et al. 2002, Rhee, Beavis et al. 2003).

Forward 5'-
AAAAAAAAAAAAAAAAAAATCTCATTACG
TACGT – 3'
Reverse 5'-
GAGCATAATTGATGACTATTATGGGCACTG
ATGAT -3'

CloneJET PCR cloning kit (Thermo ScientificTM) was used to create the gene-specific primers of the AtMYB28 (Isoforms 1, AT5G61420.2) genes for cloning into pJET1.2/blunt cloning vector (Scientific 2017). Following 30 cycles of PCR (94 0C for 30 s, 52 0C for 30 s, and 72 0C for 1.5 min), a final extension was carried out at 72 0C for 10 min. Results of PCR analysis were inserted into the pJET1.2/blunt cloning vector in the overnight incubation at

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160ºC). The recombinant plasmid containing the MYB28 gene was transformed into DH5α E. coli cells using a $CaCl_2$ chemical transformation method. Plate the transformed DH5α cells on agar plates and incubate the plate overnight at 37^0C. Selection of the positive colonies was performed according to the CloneJET PCR cloning kit (Thermo Scientific™) manual which suggests all the growing colonies were positive and contained recombinant plasmid of interest. After being transformed into Escherichia coli DH5a, competent plasmids were recovered, and the recombinant plasmid was used as a template for PCR amplification to confirm the gene.

Cloning of the MYB28 Gene in Agrobacterium Species

We have isolated the MYB28 gene from the AtMYB28- pJET1.2/blunt cloned plasmid using a template through the PCR amplification method and the reaction was performed as for the above reaction. The following set of primers were used:

Forward 5'-
AAGCTTAAAAAAAAAAAAAAAAAAATCTC
ATTACGTACGT – 3'
Reverse 5'-
TCTAGAGAGCATAATTGATGACTATTATGG
GCACTGATGAT -3'

We have to Select the pCAMBIA1301 binary vector for Agrobacterium-mediated plant transformation. We have digested the pCAMBIA1301 vector and PCR-amplified product of the MYB28 gene with the appropriate restriction enzymes (HindIII and XbaI). T4 DNA Ligase was used to insert the MYB28 gene into the pCAMBIA1301 vector at a specific cloning site into vector. The resulting recombinant plasmid carries the MYB28 gene under the control of suitable regulatory elements. We have electroporated the recombinant plasmid into Agrobacterium tumefaciens and the transformed Agrobacterium cells were poured on a plate having selective media containing 50µg Kanamycin antibiotics to select colonies carrying the MYB28 plasmid. We confirmed the presence of the MYB28 gene in the transformed Agrobacterium strains using PCR amplification techniques.

Plant Transformation: Culture the selected Agrobacterium strains under optimal conditions to achieve the desired cell density for plant transformation. We have Performed Agrobacterium-mediated plant transformation by co-cultivating target plant tissue explants (leaf discs) with the transformed Agrobacterium carrying the MYB28 gene. We have identified transgenic plant tissues that have successfully integrated the MYB28 gene by using selective media or other markers carried by the recombinant plasmid for screening. The Analysis of transgenic plants confirmed the presence and expression of the MYB28 gene by employing techniques of real-time PCR gene expression analysis.

Expression of AtMYB28 gene into Arabidopsis thaliana using RT PCR Total RNA content was extracted from several organs of A. thaliana "Chii-fu" using the AtMYB28 transcription factor RNeasy mini kit (Beltrame, Cortes et al., 2015) for expression analysis. RNase-free DNase I was then used to remove the contaminated genomic DNA (Ayukawa, Sakai et al. Using an AMV one-step RT-PCR kit, one microgram of total RNA was utilized as the template for RT-PCR (Nagura-Ikeda, Imai et al. 2020). In the 50-UTR and 30-UTR sections, respectively, primers are constructed for member-specific detection of AtMYB28s mRNA expression. For all expression investigations, the At Actin gene primer was used as a control. 35 cycles of denaturation (94 ºC for 30 s), annealing (52 ºC for 30 s), and extension (72 ºC for 1.5 min) followed by an extension at 72 ºC for 10 min comprised the PCR preparation. Pre-denaturation took place at 94 ºC for 5 minutes. The PCR products were evident on an ethidium bromide-stained 1.2% agarose gel. To guarantee experimental fidelity, two duplicates were used for the RT-PCRs. The microarray and unigene databases (http://nabic.rda.go.kr) were used to examine the AtMYB28 gene expression profile linked to the developmental phases in A. thaliana.

Chlorophyll content analysis:

Leaf, 1 g was separately chopped into small pieces and thoroughly mixed with pestle and mortar (Arnon 1949). 20 ml of 80% acetone-containing solution and 0.5gm of MgCO3 powder were applied onto the leaf. The materials were carefully stacked. The sample was then cooled down at a temperature of 40 degrees Celsius for 4 h. Thereafter, the test was whirled at 500 rpm for 5 min. The liquid was transferred into a 100-liter container. The liquid level was increased to 100 ml by adding 80% more acetone. The color of the action was tested using a machine called a spectrophotometer, which measures how much

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light is absorbed by the substance. It used light at two different wavelengths, 645nm, and 663nm, to do the test. Acetone (80%) was used in the experiment as a clear liquid (Prashanti, Sreevani et al. 2022).

The following formulas were used to estimate chlorophyll:

Chl a= 11.75×A662.6 – 2.35×A645.6

Chl b=18.61×A645.6 – 3.96× A662.6

Where Ca and Cb are the chlorophyll a and chlorophyll b, A is absorbance.

III. RESULTS AND DISCUSSION

Cloning of the MYB28 gene from Arabidopsis thaliana in Escherichia coli (E. coli) and Agrobacterium

The MYB28 gene is approximately 1899 base pairs (bp) long in Arabidopsis thaliana. This length includes the entire coding sequence (exons) and non-coding regions (introns) within the gene. The coding region is 1101bp long and encodes a polypeptide of 366 amino acids long and a molecular weight of 41.1 KDa.

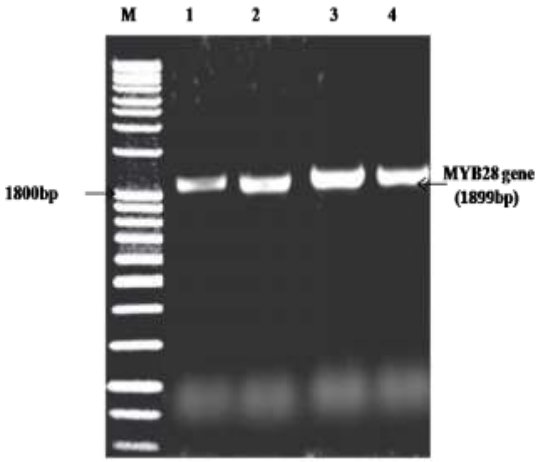

Figure 1. Cloning of A. thaliana MYB28 gene into pJET1.2/blunt cloning vector in DH5α cell (E. coli) and pCAMBIA1301 cloning vector in GV3101 cell (Agrobacterium tumefaciens). M- Marker, 1- PCR amplification of AtMYB gene from Arabidopsis thaliana genomic DNA, 2- PCR amplification from AtMYB-pJET1.2 plasmid clone from E. Coli DH5α cell strain, 3 - PCR amplification from AtMYB-pCAMBIA1301 plasmid clone from Agrobacterium tumefaciens strain GV3101, 4 - Second PCR amplification from AtMYB-pCAMBIA1301 plasmid clone from Agrobacterium tumefaciens strain GV3101.

MYB28 gene expression in Arabidopsis thaliana

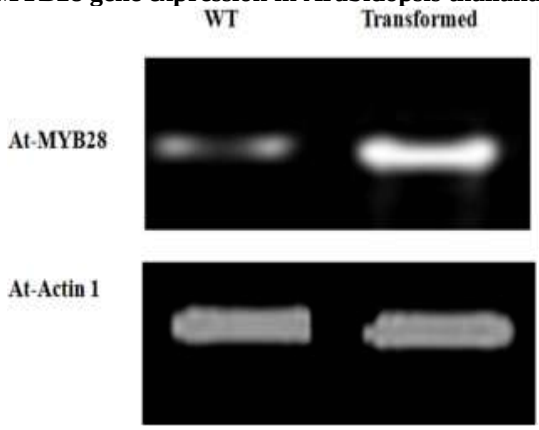

Figure 2. Expression analysis of MYB28 gene, in Arabidopsis thaliana; Wild type (WT) RT-PCR analysis of MYB28 Gene in wild type A. thaliana plant, Transformed- - RT-PCR analysis of MYB28 gene in A. Thaliana plant with an additional copy of the MYB28 gene.

Chlorophyll content in A. thaliana plant

The results of the study have been presented in Table 1. In Wild type (WT) A. thaliana young leaves the Chlorophyll a (Chlo a) and Chlorophyll b (Chlo b) contents were 1.23 µg/ml and 1.09 µg/ml, respectively. The transformed leaves contained 1.38 µg/ml of Chlo a and 1.21 µg/ml of Chlo b. Thus, our result indicates that over-expression of the MYB28 gene directly enhances the chlorophyll concentration in the A. Thaliana plant leaves (Figure 3).

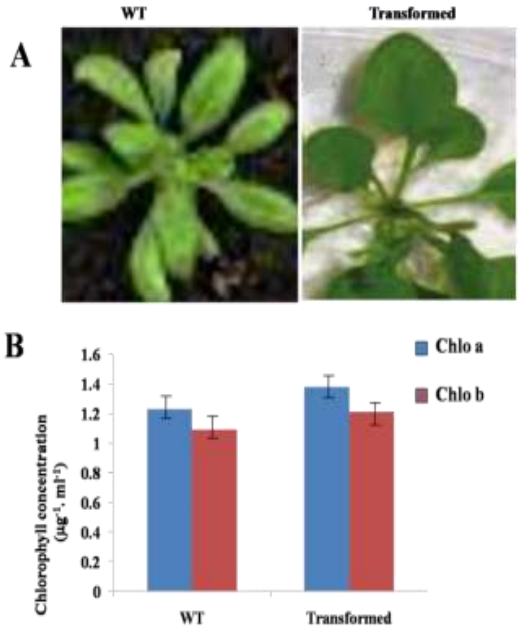

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Figure 3. Chlorophyll concentration estimation from different A. thaliana plants: (A) - Images of young plants of wild type (WT) and additional MYB28 gene containing plant (Transformed) through Agrobacterium transfection. (B)- Bar diagram showing Chlorophyll a (Chlo a) and Chlorophyll (Chlo b) concentration in wild type (WT) and additional MYB28 gene containing plant (Transformed) through Agrobacterium transfection.

Table 1. Chlorophyll concentration in different Plants of A. thaliana in µg/ml.

Types of Chlorophyll	WT Plant	Transformed plants
Chlo a	1.23 µg/ml	1.38 µg/ml
Chlo b	1.09 µg/ml	1.21 µg/ml

IV. CONCLUSION

Cloning the MYB28 gene from Arabidopsis thaliana in E. coli and Agrobacterium is a powerful approach that unlocks the potential of this transcription factor for research and biotechnological applications. The recombinant-produced MYB28 protein can be used for in vitro studies to elucidate its role in gene regulation, particularly in the context of glucosinolate biosynthesis. E.coli from plants, the MYB28 produced by coli can be used as a useful tool to engineer glucosinolate pathways in plants to improve their resistance to herbivorous animals and disease. It enables the production of MYB28 protein for functional studies, structural insights, and biotechnological advancements, ultimately contributing to a better understanding of plant defence mechanisms and potential crop improvements.

Cloning of the MYB28 gene in Agrobacterium species enables its transfer into target plants for studying its effects on glucosinolates biosynthesis and other aspects of plant biology. Cloning and expressing this gene in Agrobacterium lays the foundation for valuable research in plant defence mechanisms and potential biotechnological applications in crop improvement similarly cloning and expressing the MYB28 gene from Arabidopsis thaliana in Agrobacterium have unlocked a world of opportunities in plant genetic engineering. This breakthrough holds immense promise for enhancing crop protection, improving crop quality, and advancing our understanding of plant defence mechanisms through genetic engineering. By bridging the gap between these two host organisms, researchers and biotechnologists can harness the full potential of MYB28 for a more resilient and sustainable agriculture future.

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REFERENCES

[1]. Arnon, D. I. (1949). "Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris." Plant physiology 24(1): 1.

[2]. Ayukawa, S., Y. Sakai and D. Kiga "Aptazyme-based molecular device that converts a small-molecule input to an RNA output."

[3]. Beltrame, C., M. Côrtes, P. Bandeira and A. Figueiredo (2015). "Optimization of the RNeasy Mini Kit to obtain high-quality total RNA from sessile cells of Staphylococcus aureus." Brazilian Journal of Medical and Biological Research 48(12): 1071-1076.

[4]. Garcia-Hernandez, M., T. Berardini, G. Chen, D. Crist, A. Doyle, E. Huala, E. Knee, M. Lambrecht, N. Miller and L. A. Mueller (2002). "TAIR: a resource for integrated Arabidopsis data." Functional & integrative genomics 2: 239-253.

[5]. Hirai, M. Y., K. Sugiyama, Y. Sawada, T. Tohge, T. Obayashi, A. Suzuki, R. Araki, N. Sakurai, H. Suzuki and K. Aoki (2007). "Omics-based identification of Arabidopsis Myb transcription factors regulating aliphatic glucosinolate biosynthesis." Proceedings of the National Academy of Sciences 104(15): 6478-6483.

[6]. Nagura-Ikeda, M., K. Imai, S. Tabata, K. Miyoshi, N. Murahara, T. Mizuno, M. Horiuchi, K. Kato, Y. Imoto and M. Iwata (2020). "Clinical evaluation of self-collected saliva by quantitative reverse transcription-PCR (RT-qPCR), direct RT-

qPCR, reverse transcription–loop-mediated isothermal amplification, and a rapid antigen test to diagnose COVID-19." Journal of clinical microbiology 58(9): 10.1128/jcm. 01438-01420.

[7]. Neequaye, M., B. Steuernagel, S. Saha, M. Trick, P. Troncoso-Rey, F. van den Bosch, M. H. Traka, L. Østergaard and R. Mithen (2022). "Characterisation of the introgression of Brassica villosa genome into broccoli to enhance methionine-derived glucosinolates and associated health benefits." Frontiers in Plant Science 13: 855707.

[8]. Prashanti, B., I. Sreevani and G. Venkateswarlu (2022). "Estimation of Chlorophyll Pigment in Cyamopsis tetragonoloba (Cluster bean) in Different Soil Samples."

[9]. Rhee, S. Y., W. Beavis, T. Z. Berardini, G. Chen, D. Dixon, A. Doyle, M. Garcia-Hernandez, E. Huala, G. Lander and M. Montoya (2003). "The Arabidopsis Information Resource (TAIR): a model organism database providing a centralized, curated gateway to Arabidopsis biology, research materials and community." Nucleic acids research 31(1): 224-228.

[10]. Scientific, T. F. (2017). CloneJET PCR Cloning Kit, Consultado.

[11]. Yatusevich, R. (2008). Analysis of the MYB28, MYB29 and MYB76 transcription factors involved in the biosynthesis of aliphatic glucosinolates in Arabidopsis thaliana, Universität zu Köln.